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Molecular Defects in the Ehlers-Danlos Syndrome

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Several abnormalities in collagen biosynthesis have been described in patients with Ehlers-Danlos syndrome. Examples of collagen structural mutations as well as post-translational enzymatic defects have been detected. Patients with hydroxylysine-deficient collagen disease (Ehlers-Danlos type VI) have diminished lysyl hydroxylase activity. One mutant enzyme has been characterized which is thermally labile and had an altered affinity for ascorbate. Another mutant enzyme had a normal requirement for cofactors but activity was diminished. Type VII Ehlers-Danlos syndrome is associated with altered processing of procollagen to collagen. Most often the disorder is associated with deficient procollagen aminoprotease activity. One patient appears to represent a structural mutation of pro α_2 (I) resulting in incomplete cleavage of the amino terminal propeptide. One family with x-linked Ehlers-Danlos syndrome (type V) has been described with altered lysyl oxidase activity. Other patients with this disorder have had normal lysyl oxidase activity. The ecchymotic form of Ehlers-Danlos syndrome (type IV) has defective type III collagen synthesis. Patients have been described with absent synthesis, diminished synthesis and diminished secretion.

The Ehlers-Danlos syndrome is a striking clinical syndrome with rubber-like skin, hypermobile joints, a tendency to bruise easily, and poor wound healing [1]. In general most forms of the disorder are compatible with normal longevity; a striking exception is the ecchymotic form of Ehlers-Danlos syndrome in which premature demise occurs regularly from arterial or intestinal rupture. In 1972, the first human molecular disorder of collagen, hydroxylysine-deficient collagen disease, was described in 2 sisters with Ehlers-Danlos syndrome [2]. Since then several ultrastructural and biochemical collagen defects have been described in the Ehlers-Danlos syndrome (Table). This manuscript will focus on 4 types of Ehlers-Danlos syndrome in which biochemical collagen defects are best understood: types VI, VII, V, and IV. The range of defects is representative of the complex nature of collagen biosynthesis. These appear to include structural and regulatory collagen genomic alterations as well as intracellular and extracellular post-translation enzymatic defects.

TYPE VI EHLERS-DANLOS SYNDROME: HYDROXYLYSINE-DEFICIENT COLLAGEN DISEASE

In 1972, Pinnell et al described 2 sisters with type VI Ehlers-Danlos syndrome who had marked hyperextensibility of skin and joints, severe scoliosis, and marfanoid features [2]. Levels of hydroxylysine in skin collagen were found to be less than one per molecule of collagen and levels of hydroxylysine-derived cross-links were marked diminished [3]. The disorder is due to deficient lysyl hydroxylase activity [4] and is inherited as an autosomal recessive. Heterozygotes have intermediate activities of lysyl hydroxylase. Two mutant enzymes have been characterized. One has an altered affinity for ascorbate and is thermally labile [5]. This apparently represents a structural mutation of the enzyme. The other enzyme was kinetically normal but the activity was markedly diminished [6]. This may represent a structural or regulatory mutation.

The relative hydroxylation of tissue collagens has been variable in this disorder [2, 7]. Although skin was markedly deficient in hydroxylysine, bone was less deficient and cartilage was normally hydroxylated. The explanation for this variability is unknown although enzymatic polymorphism has not been excluded. Indeed evidence for isozymes has been reported [8]. When cell lysates from a lysyl hydroxylase deficient cell strain were tested against substrates for types I and IV collagen, preferential activity was demonstrated against type IV collagen. In one study an almost normal hydroxylysine content in skin collagen was described in association with lysyl hydroxylase deficiency [9]. Subsequent analysis of these skin fibroblasts however has revealed normal lysyl hydroxylase activity (Murad S, Pinnell SR: unpublished observations and Steinmann B: personal communcation).

The hydroxylysine content of complement component Clq was slightly low [10] or normal [11] and functional activity was unimpaired.

Therapy with large doses of ascorbate was effective in one patient with this disorder [12]. He was able to boost his urinary excretion of hydroxylysine although hydroxyproline excretion was coordinately increased. The effect appears to be due to an overall stimulation of collagen synthesis by ascorbate [13] rather than specific stimulation of lysyl hydroxylase activity. Kinetic studies of his mutant enzyme failed to detect any alteration in affinity for ascorbate [6].

TYPE VII EHLERS-DANLOS SYNDROME: ARTHROCHALASIS MULTIPLEX CONGENITA

In 1973, Lichtenstein et al reported studies on 3 type VII Ehlers-Danlos patients with a defect in conversion of procollagen to collagen [14]. These patients had short stature, hyperextensible joints, and bilateral hip dislocation. Analysis of collagen extracted from their skin revealed elongated α_1 (I) and α_2 (I) chains resulting from inefficient conversion of type I procollagen. Their cultured skin fibroblasts had deficient procollagen aminoprotease activity. Uncleaved aminoterminal propeptides apparently interfere with fibrillogenesis and intermolecular crosslinking resulting in the fragile connective tissue.

This disorder, inherited as an autosomal recessive, is biochemically similar to dermatosparaxis found in sheep [15] and cattle [16]. The striking skin fragility characteristic of dermatosparaxis however, is absent in the Ehlers-Danlos patients. The reason for this difference is not understood.

Recently another patient with type VII Ehlers-Danlos syndrome has been described with inefficient conversion of procollagen to collagen [17]. This patient has normal levels of procollagen aminoprotease. Conversion of procollagen appears to be impeded by a structural mutation in her pro α_2 (I) near the protease cleavage site. Pro α_1 (I) is normally converted but the aminopropeptide of pro α_2 (I) remains uncleaved apparently resulting in similar difficulties in fibrillogenesis found in other patients with this form of Ehlers-Danlos syndrome. This patient has, in addition to an abnormal pro α_2 (I), an equal complement of normal pro α_2 (I). She apparently represents a new structural

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Ultrastructural and biochemical collagen defects in Ehlers-Danlos syndrome

Type	Major clinical features	Ultrastructural defect	Biochemical defect
I (Gravis)	Marked joint and skin hyperextensibility. Fragile skin, poor wound healing and easy bruisability.	Variable collagen fibril diameter [29–31]	Unknown
II (Mitis)	Small joint hypermobility	Variable collagen fibril diameter [30,31]	Unknown
III (Benign hypermobile)	Large joint hypermobility	Small collagen fibril diameter [30]	Unknown
IV (Ecchymotic)	Marked bruisability, arterial and intestinal rupture	Small collagen fibril diameter [27] Dilated endoplasmic reticulum [26,27]	Diminished [25–28] or absent [23] type III collagen synthesis. Some patients may have secretion defect [26,27]
V (X-linked)	Moderate joint hypermobility. Heart valve prolapse.	Variable collagen fibril diameter [30,31]	Some patients may have lysyl oxidase deficiency [17]
VI (Hydroxylysine-deficient)	Marked joint hypermobility. Kyphoscoliosis Ocular fragility	None detected [2]	Lysyl hydroxylase deficiency [4–6]
VII (Arthrochalasis multiplex congenita)	Marked joint hypermobility Dislocated hips	Not determined	Procollagen aminoprotease deficiency [14,17] One patient with structural defect in pro α_2(I) [17]
VIII (Periodontitis)	Periodontitis Skin fragility	Not determined	Unknown
VIII (Mental retardation)	Hernias Mental retardation	Not determined	Unknown

mutation resulting in one normal and one mutant allele for pro α_2 (I).

TYPE V EHLERS-DANLOS SYNDROME: X-LINKED

In 1975, Di Ferrante et al reported studies on a patient with type V Ehlers-Danlos syndrome whose 2 male cousins were similarly affected [18]. The mothers were sisters, thus the disease was probably inherited as an X-linked recessive. Studies of skin fibroblasts in culture revealed increased collagen solubility as well as deficient lysyl oxidase activity. It should be noted that the lysyl oxidase activity in the control cell strain was minimal and the study failed to take into account the limited solubility of lysyl oxidase [19] and the striking effect of cellular density on lysyl oxidase activity [20]. Later Siegel, Black, and Bailey examined other patients with X-linked Ehlers-Danlos and found normal levels in skin of lysyl oxidase and intermolecular collagen crosslinks [21]. Subsequently Byers et al described a family with X-linked cutis laxa associated with lysyl oxidase deficiency apparently related to abnormal copper metabolism [22]. These patients had hyperextensible joints, a clinical feature more characteristic of Ehlers-Danlos syndrome than cutis laxa.

TYPE IV EHLERS-DANLOS SYNDROME

Clinical characteristics are different from other forms of Ehlers-Danlos syndrome in type IV Ehlers-Danlos syndrome. Bruising is prominent; skin is thin, transparent and fragile; tissues are not distensible; and healing may occur with hypertrophic scarring or keloids. Rupture of large arteries or perforation of gastrointestinal structures are common and life-threatening. Although autosomal dominant and autosomal recessive forms of this disorder occur, biochemical studies have been carried out only on the autosomal recessive form.

In 1975, Pope et al reported a patient with type IV Ehlers-Danlos syndrome with absent type III collagen synthesis [23]. Skin, aorta, gut, bone, and tendon were obtained shortly after death. Cyanogen bromide cleavage of these tissues revealed absence of peptides characteristic of type III collagen. Skin fibroblasts in culture failed to synthesize any detectable type III procollagen. In addition, characteristic cellular staining was absent in cultured skin fibroblasts from the patient using antisera specific for type III collagen and procollagen [24]. This patient may have had a gene deletion for type III procollagen.

Subsequent studies of fibroblasts from patients with type IV Ehlers-Danlos syndrome have revealed diminished but not absent levels of type III collagen synthesis [25, 26]. They may represent structural or regulatory mutations. Electron microscopic studies have revealed small collagen fiber diametrers [26, 27]. In 2 patients distended endoplasmic reticulum has been demonstrated in skin [28] and lung [26] fibroblasts. These fibroblasts demonstrated diminished total collagen synthesis and markedly deficient type III collagen synthesis [26, 28]. They may represent mutations which interfere with cellular secretion.

The Ehlers-Danlos phenotype is obviously associated with defective collagen structure. The variety of structural defects and abnormalities in post-translational modifications represented by the different forms of Ehlers-Danlos syndrome are characteristic of the complicated nature of collagen biosynthesis.

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